

Project No. \_\_\_\_\_

Book No. \_\_\_\_\_

TITLE \_\_\_\_\_

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Purpose: To try the new scheme in 8 days!! 10 hrs  
18 hands!!!

I Vector prep: puc 19 - 100  $\mu$ g  
0.45  $\mu$ g/ $\lambda$  picked from several tubes

restricted with Aak II ① - 1 hr = 8 hr  
Eco RI & Bam HI ② 1 hr = 3  
Aak II ③ 2 hr = 2  
successively at 37°

LT1 puc 19 100  $\mu$ g 225  $\mu$ l  
NEB buffer 4 (10x) 40  
NEB Aak II (240/ $\lambda$ ) 10  
TE 125

5  $\mu$ g round  
1.25  $\mu$ g loaded

LT1 Aak II 403 Eco RI } 20  
74121 Bam HI } 20  
100/ $\lambda$  440 → 20  $\mu$ l round after 1 hr at:  
" " " " "

LT1 Aak II 403 Eco RI } 30  
74121 Bam HI }  
100/ $\lambda$  440

5' A' C Puc 19 T-3' 50/ $\lambda$  470 2 hr at 37°  
3' T G Puc 19 A-5'

Run a, b, c on minigel along with uncut puc and  
cambion / Hind III marker.

5  $\mu$ l of a, b & c, uncut puc 0.5  $\times$  = 0.225  $\mu$ g

Final (c) stored at 4° overnight.

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Witnessed & Understood by me,

Date

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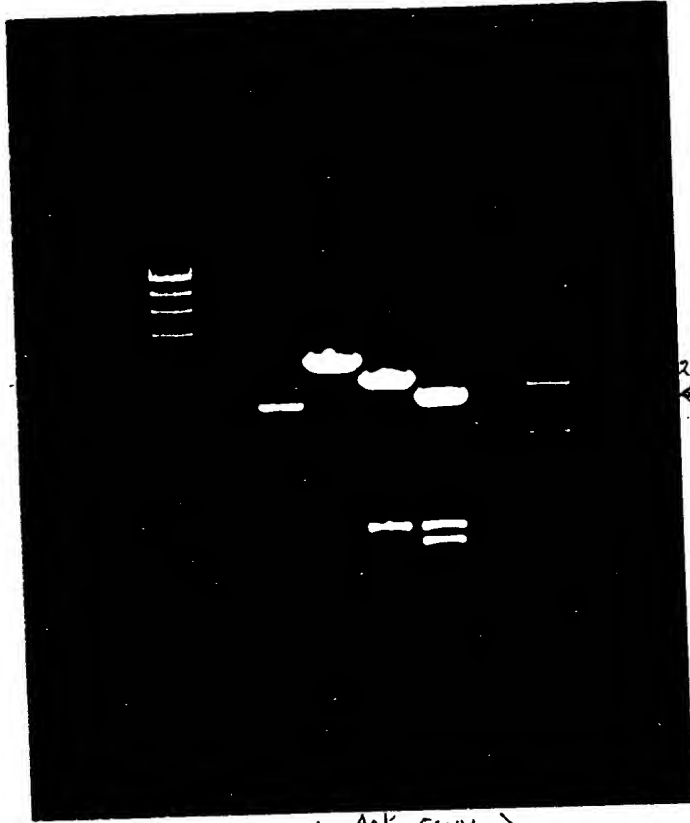
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K. Silverman

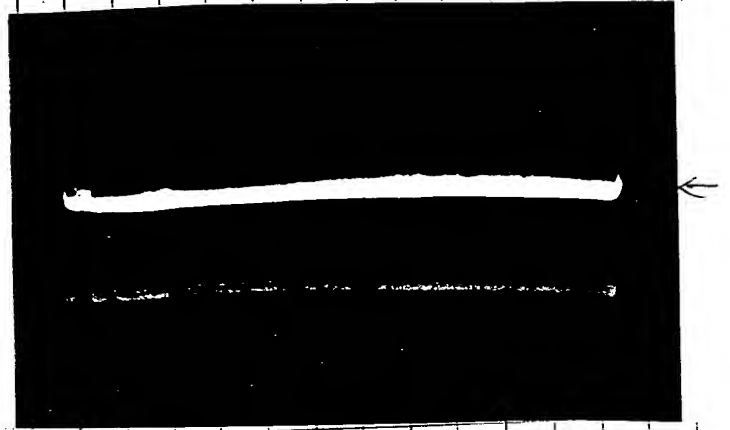
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untreated pvc    Aat I    EcoRI    BamHI    Apl 3.

- Digestion seems to be complete

- All enzymes are potent!



- loaded is 40 µg of each plate 19 and purified the vector band

- is 26.97 µg.

pvc = 2686 bp

Dat II @ 2617 + Apl III @ 806 = Vector = 1811 bp    0.6742 / 1  
 peak = 875 bp    0.325

DEAE paper containing the vector band, sealed in { 1M NaCl  
 for 10 mM Tris 8.  
 5 mM EDTA

ethanol apt (Tae) then = 100 ng / 1

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12/19/94

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12/7/94